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Localization of a β-crystallin gene, HuβA3/A1 (gene symbol: *CRYB1*), to the long arm of human chromosome 17

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Abstract. We have assigned a human β-crystallin gene, HuβA3/A1 (gene symbol: *CRYB1*), to chromosome 17 using a panel of 19 human-hamster somatic cell hybrids and blot-hybridization analysis of cell hybrid DNA. Positive probe-hybridization signal was detected in a hybrid that had lost the short arm of human chromosome 17 but retained the long arm, translocated to a hamster chromosome. In addition, in situ hybridization analysis of metaphase chromosome spreads of this cell line suggested that the most probable location for *CRYB1* is on the long arm of chromosome 17, in the region q21.

Crystallins are the major water-soluble proteins of the vertebrate eye lens. They are highly conserved in evolution and are differentially regulated during lens development (Clayton, 1974; Harding and Dilley, 1976; Piatigorsky, 1981). Recent studies at the molecular level have provided considerable insight into the understanding of the diversity, evolution, and expression of the genes encoding these lens-specific polypeptides (Piatigorsky, 1981, 1984). In mammals, crystallins can be divided into three antigenically distinct families, α, β, and γ, each of which comprises several closely related polypeptides. The β-crystallin family consists of at least six different members and is the most heterogeneous group of the three families. To provide a genetic basis for investigating the possible relationships between β-crystallins and hereditary cataracts, we have isolated and characterized one of the human β-crystallin genes (Hogg et al., 1986). This gene, designated HuβA3/A1 (gene symbol: *CRYB1*), consists of six exons, spanning approximately 8 kb (see Fig. 1). The first two exons code for an *N*-terminal extension of 32 amino acid residues, while the other four encode the four similar structural motifs of the predicted polypeptide. Transcription of HuβA3/A1 in the eye lens initiates 24 bp downstream of a TATA box, generating a single mRNA of approximately 1 kb in length. In this report, we present data showing that HuβA3/A1 is located on the long arm of chromosome 17, in the region q21.

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Materials and methods

Somatic cell hybrids

The 19 human-hamster hybrids used in this study were derived from multiple fusion experiments between various Chinese hamster auxotrophic mutant (CHO-K1) cells and different human fibroblasts or lymphoblasts. A detailed description of the human chromosome content in these hybrids has been published previously (Kao et al., 1976; Law and Kao, 1978; Lai et al., 1983; Moore et al., 1984; Inui et al., 1985). The presence or absence of human chromosome 17 in these hybrids was determined by cytogenetic methods (Alhadeff et al., 1977; Morse et al., 1982), isozyme marker

galactokinase (GALK) assay (Harris and Hopkinson, 1976), and molecular hybridization analysis (Southern, 1975) using a thymidine kinase (TK) gene probe (Lau and Kan, 1984) generously provided by Dr. Yun-Fai Lau.

DNA hybridization analysis

Total cellular DNAs were prepared from cultured human, CHO, and hybrid cell lines as described elsewhere (Gusella et al., 1979; Kao et al., 1982). Restriction endonuclease digestions were carried out according to conditions specified by the enzyme supplier (Boehringer Mannheim). Digested DNA samples were size-fractionated on 0.8% agarose gels, blotted to nitrocellulose filters (Schleicher & Schuell), and baked in vacuo (Southern, 1975).

Two probes derived from the human β-crystallin gene (HuβA3/A1) were used for blot-hybridization analysis. The first probe (probe A) was a 1.4-kb fragment spanning the third exon and its flanking sequences (Fig. 1). The second probe (probe B) was a 2.5-kb fragment corresponding to the 3′ flanking region of the gene (Fig. 1). These fragments were subcloned from the original recombinant phage clones, λ8A and λ14A, as described previously (Hogg et al., 1986). The probes used for hybridization experiments were labeled to a specific activity of approximately 2×10^8 cpm/μg DNA with [α^{32}P]-dCTP (New England Nuclear), using a nick-translation kit (Bethesda Research Lab.). Nitrocellulose filter prehybridization and hybridization were performed according to published procedures (Wahl et al., 1979).

In situ hybridization

Hybridization of HuβA3/A1 sequences to metaphase chromosomes was performed using the technique of Harper and Saunders (1981), with modifications (Zabel et al., 1983; Funderburk et al., 1984).

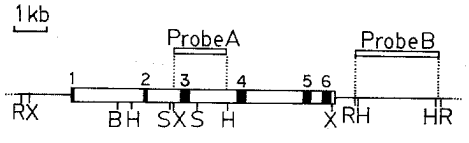

Fig. 1. Structure of HuβA3/A1 gene *(CRYB1)* showing probe-hybridizing regions. Exons, shaded boxes, are numbered 1 through 6. Probe A is a 1.4-kb *Xba*I-*Eco*RI fragment derived from recombinant phage λ8A and subcloned in a pSV2gpt-based vector. Probe B is a 2.5-kb *Eco*RI fragment derived from phase λ14A and subcloned in pUC9. The *Eco*RI site of the 1.4-kb fragment and the distal one of the 2.5-kb fragment were introduced during phage cloning (Lawn et al., 1978). R: *Eco*RI; X: *Xba*I; B: *Bam*HI; H: *Hin*dIII; S: *Sst*I.

Results

Our previous studies demonstrated that HuβA3/A1 *(CRYB1)* contains six exons spanning over 8 kb (Hogg et al., 1986). Since the introns and the flanking sequences of this gene contain numerous repetitive DNA sequences, two gene-specific probes (probes A and B) were derived for the present study (Fig. 1). Probe A detected a 2.8-kb β-crystallin-specific fragment in *Hin*dIII-digested total human DNA (Fig. 2, lane 1), whereas probe B hybridized to two *Hin*dIII fragments, a strong 2.3-kb band and a weak 4.0-kb band (Fig. 2, lane 13). The presence of other hybridizing bands in these lanes was due to contamination of plasmid DNA in the sample.

When probes A and B were hybridized to a panel of *Hin*dIII-digested DNA samples from human-hamster somatic cell hybrids, the CRYB1 sequences

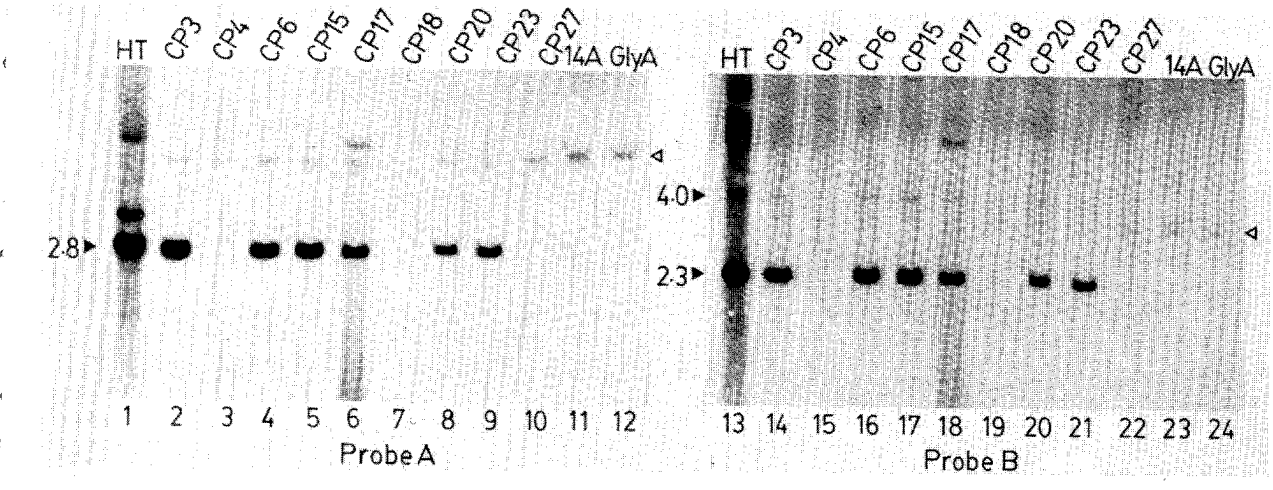

Fig. 2. Blot-hybridization analysis of human-hamster cell hybrid DNAs with HuβA3/A1 gene probes. Total DNAs from the indicated cell lines were digested with *Hin*dIII, electrophoresed on a 0.8% agarose gel, blotted to nitrocellulose filters, and hybridized with either probe A or probe B as shown. HuβA3/A1-specific bands and cross-hybridizing hamster bands are indicated by solid and open triangles, respectively. Some DNA samples were contaminated with low levels of plasmid DNA (lanes 1, 6, 13, and 18).

were clearly distinquishable from the weak hamster bands (Fig. 2, lanes 2, 4–6, 8, 9, 14, 16–18, 20, and 21). Moreover, as expected, all cell lines hybridized to either both probes or neither of them. Synteny analysis of the presence and absence of the CRYB1 sequence in 19 human-hamster somatic cell hybrids revealed complete concordance between *CRYB1* and human chromosome 17 (Table I).

Information on the regional localization of *CRYB1* on chromosome 17 was derived from hybrid CP23. Cytogenetic analyses of this cell line, using both trypsin banding and differential staining techniques, showed that it had lost the short arm of human chromosome 17 and that the long arm of this chromosome had been translocated to a hamster chromosome (Fig. 3). Since CP23 showed positive hybridizing bands with both CRYB1 probes (Fig. 2, lanes 9 and 21), we assigned *CRYB1* to the long arm of chromosome 17.

Regional localization of *CRYB1* was also performed by in situ hybridization analysis using ^{3}H-labeled probe A on metaphase chromosomes of a subclone of CP23 containing the translocated long arm of human chromosome 17 as the only apparent human chromosomal material. A total of 384 spreads were examined, and 624 grains were counted. Although most grains were randomly distributed, 20 were found on the translocated long arm of human chromosome 17. As the majority of grains were detected in the region q21 (Fig. 4), it is reasonable to conclude that this is the location for *CRYB1*. A similar conclusion could also be derived from the result of in situ hybridization analysis with metaphase chromosomes of human lymphocytes (data not shown).

Table I. Human chromosomes present in a panel of 19 human/CHO-K1 hybrids were identified by trypsin-banding and Giemsa-11 differential staining and by isozyme analysis. GALK activity was detected by isozyme electrophoresis, whereas TK and CRYB1 were scored by blot-hybridization analysis using the respective gene probes. The concordant hybrids showed cosegregation of the human chromosome and CRYB1. Concordant frequency refers to the number of concordant hybrids/total number of hybrids analyzed

Hybrid	Human chromosome																							Marker		
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	GALK	TK	CRYB1
CP3–1	−	−	−	+	+	−	−	−	−	−	+	+	−	+	−	+	+	+	+	+	+	−	+	+	+	+
CP4–1	−	−	−	+	+	−	−	+	−	−	+	−	−	+	−	−	−	−	−	−	−	+	+	−	−	−
CP5–1	+	−	−	−	+	−	−	+	+	−	−	+	−	+	+	−	+	−	+	−	+	+	−	+	+	+
CP6–1	−	−	−	+	−	−	−	−	−	−	−	+	−	+	−	−	+	+	+	+	+	+	−	+	+	+
CP12–1	−	+	−	+	−	−	−	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+	+	−	−	−
CP14–1	−	−	−	+	+	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	+	+	+	+	+	+
CP15–1	−	−	−	+	+	−	−	−	−	−	+	+	−	−	+	+	+	−	−	−	−	−	−	+	+	+
CP16–1	−	−	−	−	+	−	−	−	−	−	−	−	−	+	−	−	+	−	+	+	+	+	−	+	+	+
CP17–1	+	−	−	+	+	−	−	−	−	−	−	+	−	−	−	−	+	−	−	−	−	−	−	+	+	+
CP18–1	+	−	−	−	−	−	−	+	−	−	+	−	−	+	+	−	+	+	+	−	−	−	−	+	+	+
CP20–1	−	+	−	−	+	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	+	+
CP26–1	+	−	−	+	+	+	+	−	+	+	+	+	+	−	−	−	+	−	−	−	−	+	−	+	+	+
CP28–1	+	−	−	+	+	−	−	+	+	−	−	−	−	−	−	−	−	+	+	+	−	−	−	−	−	−
CP29–1	−	+	+	+	+	−	−	−	−	−	+	+	−	+	+	+	−	+	+	−	+	+	+	−	−	−
CP23–1	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	+[a]	−	−	−	−	−	−	+	+	+
CP43	−	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−
CP38–1	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
J1	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
12A	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−
Number of concordant hybrids	11	7	7	10	13	9	9	7	8	8	8	11	8	11	10	9	19	9	11	9	13	9	7			
Concordant frequency (%)	57.9	36.8	36.8	52.6	68.4	42.1	47.4	36.8	47.4	42.1	42.1	57.9	42.1	57.9	52.6	47.4	100	47.4	57.9	47.4	68.4	47.4	36.8			

[a] Only the long arm of chromosome 17 is present.

Discussion

The localization of HuβA3/A1 *(CRYB1)* to the long arm of human chromosome 17 provides further insight into the evolution of the β- and γ-crystallin gene families. Because these two classes of polypeptides show significant identity at the amino acid sequence level, it has been proposed that they constitute members of a βγ superfamily (Piatigorsky, 1984). However, recent structural analyses of the genes encoding these proteins suggest that the β- and γ-crystallin genes diverged much earlier than individual members of the two families evolved (Moormann et al., 1983; Schoenmakers et al., 1984; Meakin et al., 1985; Hogg et al., 1986). Consistent with this hypothesis, the γ-crystallin gene cluster *(CRYG)* maps to the long arm of human chromosome 2 (Dunnen et al., 1985; Willard et al., 1985; Shiloh et al., 1986), a location different from that of *CRYB1*.

Previous analysis of the different β-crystallins from bovine lenses has revealed significant divergence among this group of proteins (Berbers et al., 1984). The distant homology among different β gene members is also reflected by the weak cross-hybridization between different β gene probes (Inana et al., 1982; Hejtmancik et al., 1984; Schoenmakers et al., 1984) although the genes appear to have a similar structure (Schoenmakers et al., 1984; Hogg et al., 1986). Moreover, analysis of overlapping genomic clones failed to show clustering of the β genes (Schoenmakers et al., 1984). In contrast, all γ-crystallin genes share a high degree of sequence homology and are tandemly arranged in a head-to-tail conformation within the genome (Moormann et al., 1984, 1985; Schoenmakers et al., 1984; Meakin et al., 1985). Therefore, the tandem duplication of the γ genes probably occurred more recently than the emergence of the different β genes.

Because crystallins are generally believed to be the templates of lens transparency, these proteins have been extensively analyzed for their possible role in cataractogenesis (Harding and Dilley, 1976; Harding, 1981). Alterations in the structure of these proteins or their relative levels have been frequently detected in cataractous lenses, although, as yet, no direct correla-

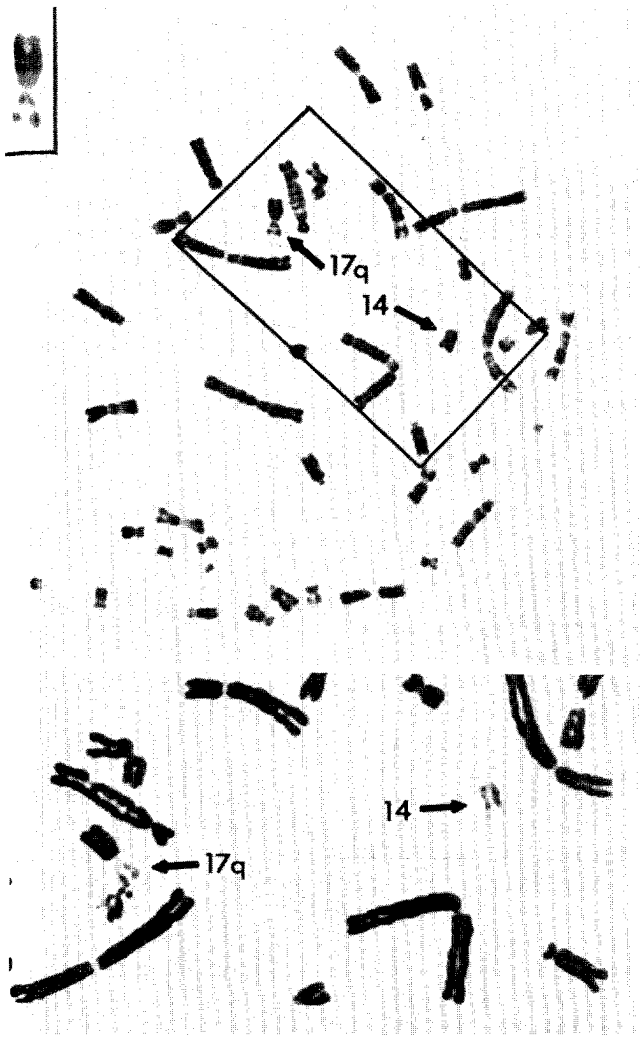

Fig. 3. Cytogenetic analysis of human/CHO-K1 hybrid CP23. The same slide was sequentially stained with trypsin (upper panel) followed by Giemsa-11 (lower panel). This Giemsa-trypsin banded metaphase shows the presence of two human chromosomes, 14 and 17q; the latter has been translocated to a Chinese hamster chromosome. An enlargement of this chromosome is shown in the upper left-hand corner. The lower panel corresponds to the boxed area shown in the upper panel. Arrows indicate human chromosomal material that was identified by sequential staining.

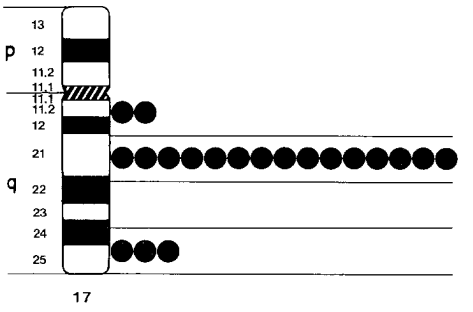

Fig. 4. Distribution of silver grains indicating the regional localization of *CRYB1* on the long arm of human chromosome 17. Scoring of grains was performed using a subclone of hybrid cell line CP23 that contained the translocated long arm of chromosome 17 as the only detectable human chromosomal material.

tion can be drawn between these changes and possible mutations in any of the three crystallin gene families. The chromosomal localization of various crystallin genes thus provides a means for studying the hereditary forms of cataracts in the human population (McKusick, 1984). Thus far, crystallin genes have been assigned to at least four different locations within the human genome. The two α-crystallin genes, αA and αB, map to human chromosome 21 and 16, respectively (Quax-Jeuken et al., 1985), whereas the γ-crystallin gene cluster is located on the long arm of chromosome 2 (Dunnen et al., 1985; Willard et al., 1985; Shiloh et al., 1986). Here we have provided evidence that one of the β-crystallin genes resides on the long arm of chromosome 17. These data, in conjunction with mapping studies on hereditary cataract disorders, should therefore facilitate identification of mutations associated with any of these genes. Furthermore, it is now possible to perform linkage analyses on families with cataracts, using restriction fragment length polymorphisms associated with each of these gene loci.

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